

Clinical outcome following anterior cervical discectomy and fusion with and without anterior cervical plating for the treatment of cervical disc herniation—a 25-year follow-up study

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Abstract Extreme long-term clinical outcome studies following anterior cervical discectomy and fusion (ACDF) with an autologous iliac crest with and without Caspar plating (ACDF + CP) for the treatment of radiculopathy caused by cervical disc herniation (CDH) are extremely rare. Hospital records of patients who underwent ACDF or ACDF + CP for the treatment of CDH at least 17 years ago were reviewed. Information about diagnosis, surgery, pre- and postoperative clinical process, and repeated procedure was analyzed. At final follow-up, patients were reviewed with a standardized questionnaire including the current neurological status, Neck Disability Index (NDI), Odom's criteria, a modified EQ-5D, and limitations in quality of life. One hundred twenty-two patients with a mean follow-up of 25 years were evaluated. ACDF was performed in 80 and ACDF + CP in 42 patients, respectively. At final follow-up, 81.1% of patients were free of radicular pain and had no repeated procedure. According to Odom's criteria, 86.1% of good to excellent functional recovery was noted. The mean NDI and EQ-5D was 14% and 5 points, respectively. There was no significant difference in the assessed clinical outcome parameters between patients treated with ACDF and ACDF + CP. The rate for repeated procedure due to degenerative cervical disorders was 10.7 and 7.4% due to symptomatic adjacent segment disease with 25 years.

ACDF and ACDF + CP achieved a high rate radicular pain relief (89.3%) and clinical success (86.1%) for the treatment of CDH within a 25 years follow-up. No statistical difference concerning clinical outcome and rate of repeated procedure was detected.

Keywords ACDF · Adjacent segment disease · Cervical plate · Cervical disc herniation · Clinical outcome, long-term follow-up

Introduction

Degenerative cervical disc disease (DCDD) is the cause for 20 to 30% of all patients with pain of the vertebral column [14]. Anterior cervical discectomy and fusion (ACDF) has become an advanced and widely adopted technique for the treatment of cervical radiculopathy and myelopathy since it was first described in the 1950s [10, 37]. ACDF has a likelihood of 90% for relief of radicular complaints such as pain [3–5, 20]. Despite its high acceptance and success, ACDF performed with an autologous iliac crest graft is associated with several complications such as graft subsidence, graft dislocation, segmental pseudarthrosis, and morbidity at the iliac crest donor side (ICDS) [8].

Fusion rates for non-instrumented one-level ACDF with autograft have been reported to be 83–99%. The process of fusion is influenced by comorbidities and number of operated segments. Fusion rates may drop down in multi-level ACDF without instrumentation [2, 9]. ACDF with cervical plating (ACDF + CP) has been recommended to reduce graft associated complications [7, 8]. Further, ACDF + CP has been recommended for two or more level procedures, for instability, for kyphosis, and for patients with smoking history and

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diabetes. However, adding of a cervical plate is also associated with plate-related complications such as material failure and additional cost. Especially in one-level procedures, it is discussed controversially. Additionally, long-term follow-up series have raised concerns about degeneration of the segments adjacent to the fused level. In the past decades, cervical spine fusion procedures have increased constantly [12, 27, 34]. Degenerative findings do not necessarily correlate with clinical and functional outcomes of patients. Therefore, the authors will use the term of “symptomatic adjacent segment disease” (sASD) to refer to the development of new symptoms that correlate to radiographic degenerative findings at the adjacent segment.

Currently, it remains unclear whether ACDF + CP is superior to ACDF or not for the treatment of CDH.

To our knowledge, there is no published study which analyzes and compares the clinical outcome and the development of sASD after ACDF and ACDF + CP for the treatment of CDH in a follow-up of 25 years.

Material and method

Patient population

We retrospectively reviewed files of patients with CDH, who had undergone a de novo 1- to 3-level Smith-Robinson

procedure with an autologous iliac crest graft (AICB) with or without additional Caspar plate fixation at our neurosurgical department at a minimum of 17 years ago. A detailed breakdown of all operated segments is summarized on Fig. 1. All documents of the initial procedure (ACDF or ACDF + CP) and all documents in case of repeated procedures were thoroughly reviewed.

Inclusion criteria were patients with full documentation of the preoperative neurological status, of the detailed procedure note, of the postoperative process during hospitalization, of the postoperative neurological status at hospital discharge, and of full contact detail information. A flow chart summarizes the demographics of all patients who participated in the study [Fig. 2].

Preoperative and postoperative neurological status and questionnaire

Each patient’s file was reviewed thoroughly. The data concerning the preoperative and postoperative neurological status is based on the documentation in the patient’s file with special focus on symptoms which are associated with CDH, such as radicular pain, neck pain, motor dysfunction, and sensory dysfunction. The diagnosis was made by physical examination, myelography, computed tomography–myelography, and magnetic resonance imaging.

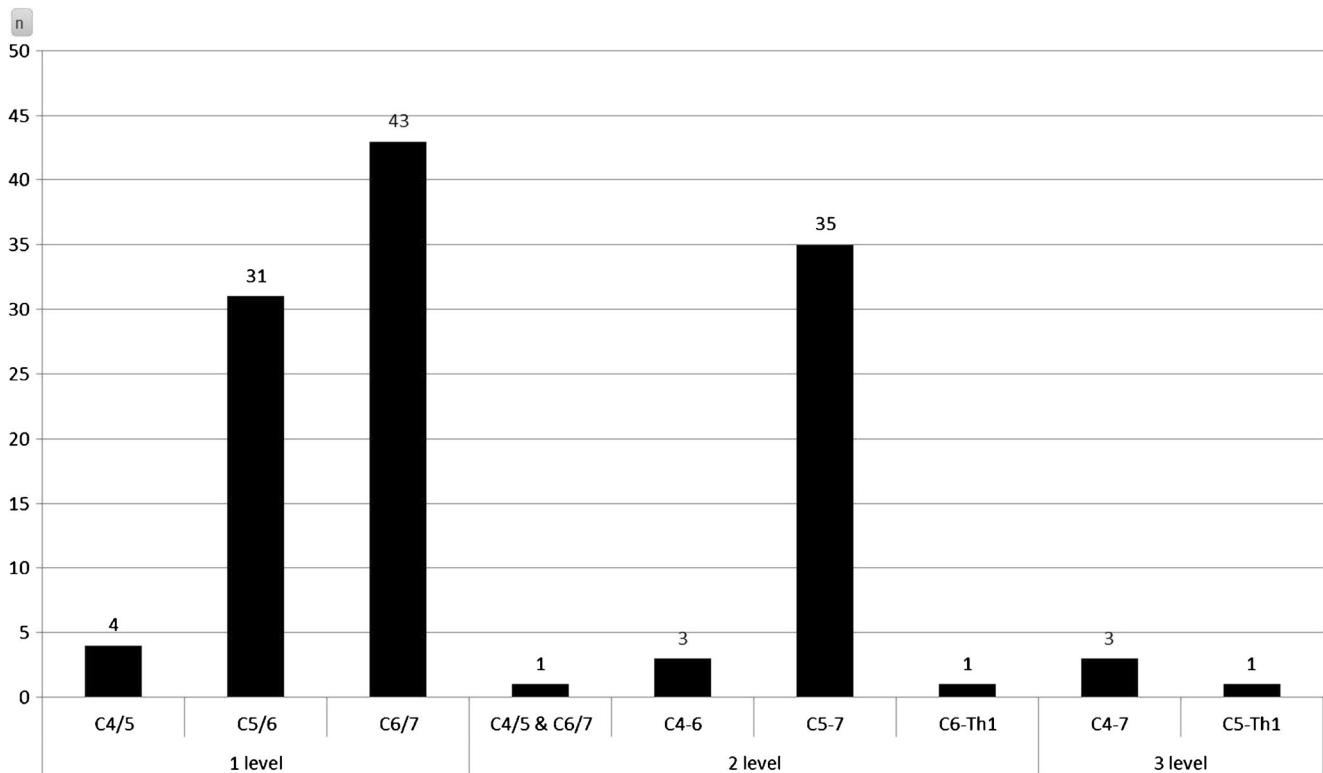


Fig. 1 A detailed breakdown of all operated segments

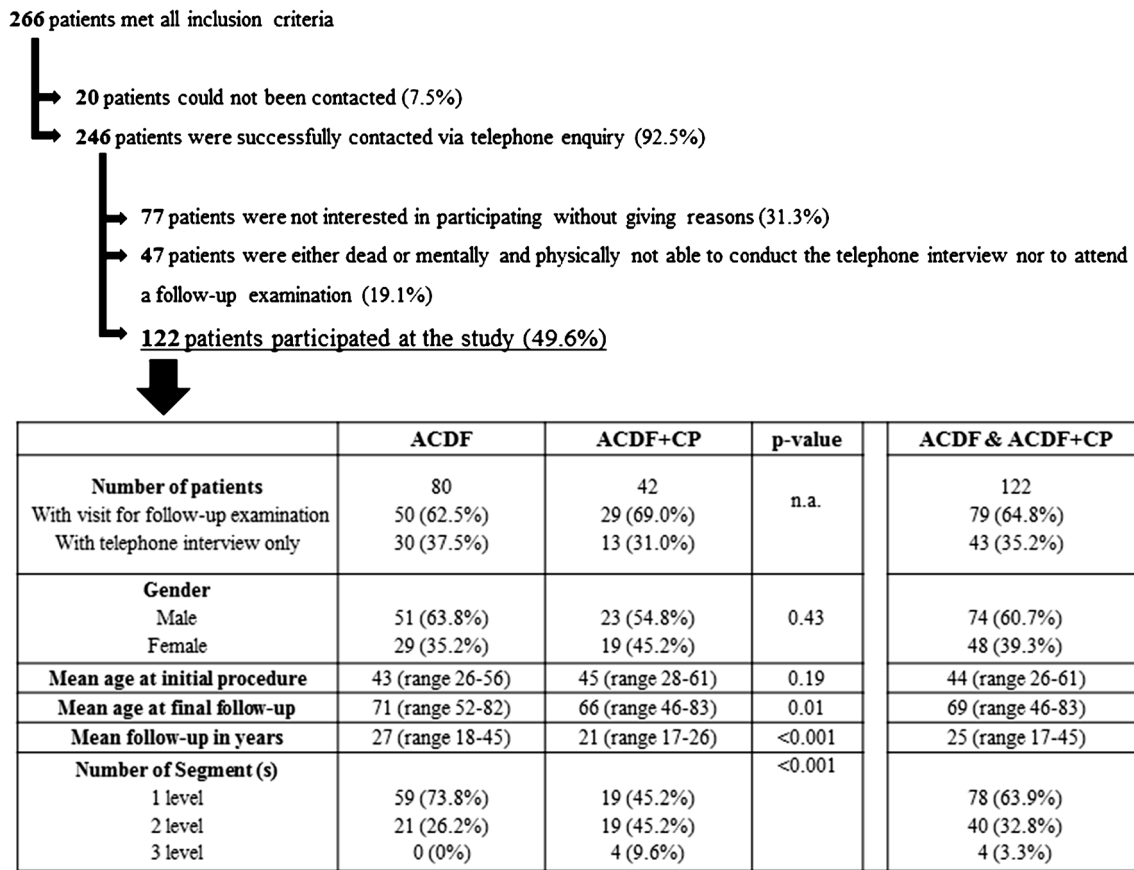


Fig. 2 A summary of the demographics of all patients who participated in the study

The data concerning the neurological status at final follow-up is based on the telephone interview and the personal examination. A detailed telephone interview was performed to inquire the clinical course of each patient with special focus on pre- and postoperative courses concerning radicular pain and neck pain which was assessed by numeric pain rating scale (NRS) and postoperative improvement concerning motor and sensory function. A standardized questionnaire was used to assess the clinical outcome according to Neck Disability Index (NDI) [42], Odom’s criteria [30], and via five questions of the EQ-5D questionnaire [Table 1]. Clinical success was defined as excellent and good clinical outcome according to Odom’s criteria. Furthermore, each participant was asked about limitations in their daily life due to ICDS discomfort and dysphagia.

In addition to the telephone interview and questionnaire, a personal examination was offered to each patient.

Statistical analysis

The SPSS statistical software package (SPSS, Inc., Chicago, IL) was used for statistical analysis of the data. We used Fisher’s exact Tests and the Fisher–Freeman–Halton exact Test to compare relative frequencies of a binary outcome

and an outcome with more than two categories between two independent groups, respectively. The

Wilcoxon was used to compare non-parametric paired sample tests and the Mann–Whitney *U* test was applied for the comparison of a quantitative non-normal outcome between two groups. Any *p* values given were two sided. We used the Kaplan–Meier survival analysis and Mantel–Cox test for comparison of repeated procedures for both groups. A *p* value of <0.05 was assumed sufficient to indicate statistical significance.

Results

There were no significant differences regarding the mean age at initial procedure and gender between patients who underwent ACDF and the patients who underwent ACDF + PS. There were significant differences regarding the mean age at follow-up, the length of follow-up, and the number of operated segments between the patients who underwent ACDF and the patients who underwent ACDF + CP (Fig. 2). There were no significant differences regarding age, gender, and operated segments between the patients who participated in the study and the patients who were lost at follow-up (Mann–

Table 1 EQ-5D questionnaire and results

EQ-5D questionnaire		Results (%)
Mobility		
Level 1	I have no problems in walking about	90.2
Level 2	I have some problems in walking about	9.0
Level 3	I am confined to bed	0.8
Self-care		
Level 1	I have no problems with self-care	93.4
Level 2	I have some problems washing or dressing myself	5.7
Level 3	I am unable to wash or dress myself	0.8
Usual activities (e.g. work, study, housework, family, or leisure activities)		
Level 1	I have no problems with performing my usual activities	85.2
Level 2	I have some problems with performing my usual activities	13.1
Level 3	I am unable to perform my usual activities	1.6
Pain/discomfort		
Level 1	I have no pain or discomfort	92.6
Level 2	I have moderate pain or discomfort	6.6
Level 3	I have extreme pain or discomfort	0.8
Anxiety/depression		
Level 1	I am not anxious or depressed	95.9
Level 2	I am moderately anxious or depressed	4.1
Level 3	I am extremely anxious or depressed	0.0

Whitney U test $p = 0.086$, two-sided Fisher's exact test $p = 0.1551$, Fisher–Freeman–Halton exact test $p = 0.6221$).

There were no significant differences regarding age, gender, and operated segments between patients who answered the questionnaire and patients who answered the questionnaire and presented for a personal clinical examination (Mann–Whitney U test $p = 0.177$, two-sided Fisher's exact test $p = 0.8465$, Fisher–Freeman–Halton exact test $p = 0.6302$).

Preoperative and postoperative neurological status and results of questionnaire

A detailed compilation of preoperative, postoperative, and final follow-up clinical outcomes is shown in Table 1 and Table 2.

Preoperatively, the median NRS score for radicular pain was 8 (range 3–10), the median NRS score for neck pain was 4 (range 2–9).

Postoperatively, in two patients, no improvement of radicular pain and neck pain was documented (1.6%); one patient had a new deltoid paresis was documented (0.8%) and six patients reported a new onset of sensory disturbance (4.9%).

At final follow-up, the median NRS score for radicular pain was decreased significantly to 2 (range 0–8) (Wilcoxon test $p < 0.001$). Thirteen patients reported about intermittent radicular pain or neck pain (10.7%) and three among those patients had a second procedure due to DCDD (23.1%).

Eight out of those 13 patients had none (61.5%), four patients had minor (30.8%), and one patient had serious limitations in their daily activities (7.7%).

Concerning pain at the iliac crest donor side (ICDS), the mean time period of discomfort was reported to be 1.8 months (range 0–24 months). None of the patients reported to have dysphagia.

Currently, three patients reported to take pain medication temporarily due to discomfort at the ICDS (1.6%). Ten patients reported about smaller limitations in their daily life due to problems at the bone harvest site (9.8%).

Neurological examination

Seventy-nine (64.8%) patients attended a physical examination for final follow-up at our department. At follow-up, none of the patients reported radicular pain. A preoperative motor deficit was documented in 58 of patients (73.4%); among those, a residual paresis was documented in eight patients at discharge (17.2%). At final follow-up, one patient had a mild residual motoric deficit (1.7%). At final follow-up, one patient presented with new developed gait disturbance (1.3%).

A preoperative sensory deficit at a specific radicular distribution was documented in 70 patients (88.6%); among those, 12 patients had a residual sensory deficit at discharge (17.1%). Postoperative five patients reported to have a new sensory deficit (4.8%). At follow-up examination, an improved but

Table 2 Patient clinical outcome

		ACDF	ACDF + CP	<i>p</i> value	ACDF and ACDF + CP	
Preoperative	Radicular pain	79 (98.8%)	42 (100%)	1.00	121 (99.2%)	
	Neck pain	52 (65.0%)	22 (52.4%)	0.24	74 (60.7%)	
	Motor deficit	60 (75.0%)	22 (52.4%)	0.01	82 (67.2)	
	Sensory disturbance	71 (88.8%)	37 (88.1%)	1.00	108 (88.5%)	
Postoperative	Free of radicular pain	16 (20.3%)	14 (33.3%)	0.12	30 (24.8%)	
	Free or improvement of radicular pain	78 (98.7%)	41 (97.6%)	1.00	119 (97.5%)	
	Complete recovery of motor deficit	47 (78.3%)	20 (90.9%)	0.33	67 (81.7%)	
	Complete recovery or improvement of motor deficit	57 (95.0%)	21 (95.5%)	0.27	78 (95.1%)	
	Complete recovery of sensory disturbance	56 (78.8%)	29 (78.4%)	1.00	85 (78.7%)	
	Complete recovery or improvement of sensory disturbance	70 (98.5%)	35 (94.6%)	0.27	105 (97.2%)	
	Final follow-up	Free of radicular pain	70 (87.5%)	39 (92.8%)	0.53	109 (89.3%)
	Free of radicular pain and no repeat procedure due to DCDD	62 (77.5%)	37 (88.1%)	0.22	99 (81.1%)	
Final follow-up	Mean NDI in %	14 (range 2–44%)	14 (range 5–52%)	0.97	14 (range 2–52%)	
	minimal disability 0–20%	67 (54.9%)	36 (85.7%)		103 (88.4%)	
	moderate disability 21–40%	11 (9.0%)	5 (11.9%)		16 (13.1%)	
	severe disability 41–60%	2 (1.6%)	1 (2.4%)		3 (2.4%)	
	Mean score of EQ-5D questionnaire	5 (range 5–14)	5 (range 5–10)	0.75	5 (range 5–14)	
	Clinical success according to Odom's criteria	70 (87.5%)	35 (83.3%)	0.58	105 (86.1%)	
	Repeat procedure due to DCDD	11 (13.8%)	2 (4.8%)	0.21	13 (10.7%)	
	Repeat procedure due to symptomatic ASD	8 (10.0%)	1 (2.4%)	0.09	9 (7.4%)	

minimal sensory disturbance remained in 11 out of those 12 patients (15.7%).

Seven patients reported to have mild neck pain while head motion (8.9%). Two patients reported neck pain which is the cause for minor problems in their daily life (2.5%).

Patients with repeated procedure

According to the file, the operative report, and the telephone interview, 21 out of 122 patients had repeated procedure (17.2%).

Four patients underwent repeated procedure within the first week after surgery. Another four patients developed chronic dysphagia and their CP was removed. In three patients, excessive scar formation was identified, and in one case, a true implant failure with a broken plate was noted. In none of the cases was any injury of the esophagus or a screw dislocation documented.

Thirteen patients had repeated procedure at the cervical spine (10.7%). Among those, nine patients had repeated procedure due to symptomatic ASD (7.4%), three patients had repeated surgery at the second segment cranial to the index ACDF (2.4%). In one case each, a dorsal approach for laminectomy, an artificial disc replacement, and ACDF + CP was performed 22, 25, and 27 years after index surgery, respectively. Finally, in one patient, resection of a ventral

formation of spondylophytes was performed 25 years after the index ACDF (0.8%), Table 3.

There was no significant difference in the rate of repeated procedure for sASD (Mantel–Cox–test $p = 0.170$) and in the rate for repeated procedures for degenerative disease (Mantel–Cox–test $p = 0.396$) at the cervical spine between ACDF and ACDF + CP [Figs. 3 and 4].

Discussion

ACDF has become a widely adopted technique and is considered the gold standard to treat cervical radiculopathy and myelopathy. However, some authors even propagate that clinical outcome may not be affected in the event of non-union and that patients may remain asymptomatic [25].

Cervical spondylosis is a process of degeneration defined as “vertebral osteophytosis secondary to degenerative disc disease.” In the early stages, the elasticity of the disc decreases, while in the latter stages, segmental kyphosis and compression of vascular and/or neural structures occur. Therefore, CDH occurs in one of the earlier stages were as progressive kyphosis and cervical spondylotic myelopathy are the endpoints of this progress [16].

In the present study, the clinical success rate according to Odom's criteria is similar to the rate of patients who reported

Table 3 Repeated procedures

	No. of patients	Surgical technique of repeated procedure	Diagnosis at repeated procedure	Years after initial procedure
Procedure after one-level ACDF				
Index and 1 segment above	1	ACDF + PS	Pseudarthrosis at index segment with kyphotic malalignment and adjacent cervical disc herniation	4
1 segment above	1	ACDF	Cervical disc herniation	6
1 segment below	1	ACDF	Cervical disc herniation	8
	1	ACDF + PS	Cervical spondylosis	4
	1	ACDF + PS	Cervical disc herniation	12
2 segments above	1	ADR	Cervical disc herniation	25
Iliac crest donor side	1	Osteosynthesis at the spina iliac anterior superior	Hip fracture	^a
Iliac crest donor side	1	Evacuation of hematoma	Postoperative bleeding	^a
Procedure after two-level ACDF				
1. segment above	1	ADCF	Cervical disc herniation	7
	1	ACDF + PS	Cervical disc herniation	1
1 and 2 segments above	1	ACDF + PS	Cervical spondylosis	26
	1	Resection of ventral spondylophytes	Spondylophitic formation	25
2 segments above	1	ACDF + PS	Instability	27
At caudal index level	1	Repositioning of iliac crest graft	Dislocation of iliac crest graft	^a
Procedure after one-level ACDF + PS				
Removal of cervical plate	1	Cervical plate removal	Chronic dysphagia	14
	1		Broken plate	2
Procedure after two-level ACDF + PS				
1 segment above	1	ACDF	Cervical disc herniation	12
2 segments above	1	Laminectomy	Cervical stenosis due to hypertrophy of ligamentum flavum	22
Removal of cervical plate	1	Removal of cervical plate	Chronic dysphagia	6
Iliac crest donor side	1	Wound debridement	Postoperative wound infection	^a
Procedure after three-level ACDF + PS				
Removal of cervical plate	1	Removal of cervical plate	Chronic dysphagia	2

^a Procedures within 1 week after ACDF/ACDF + PS

Fig. 3 Time for repeated procedure for sASD

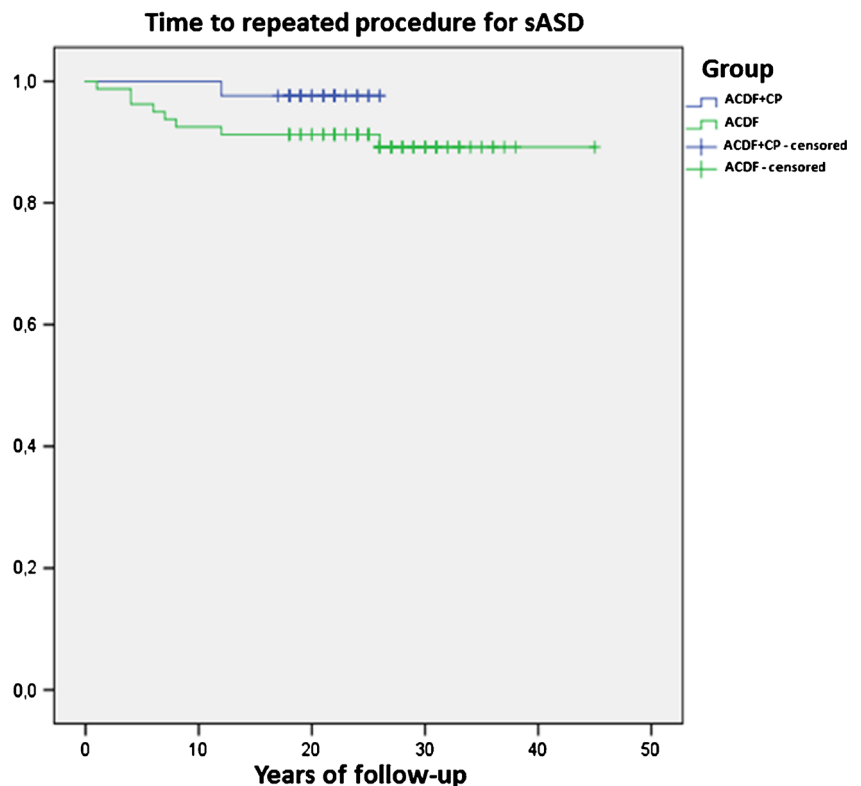
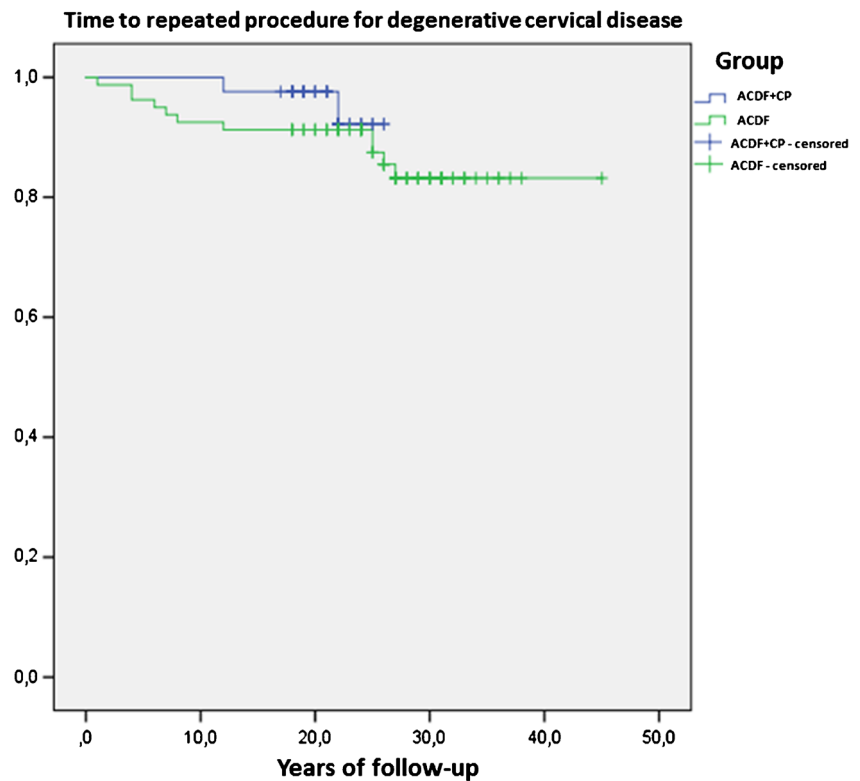


Fig. 4 Time for repeated procedure for degenerative cervical disease



to be free of radicular pain at final follow-up. These results are comparable to other studies who reported 78–86%, even up to 100% good and excellent clinical outcome [6, 13, 15, 33, 39].

However, the present study also demonstrates that the rate of patients who were free of pain or with almost no pain at discharge decreases from 97.5 to 81.1% at final follow-up. The authors believe that several factors contribute to this finding such as the natural degeneration of the cervical spine and graft subsidence with concomitant neuroforaminal narrowing. Further, the rate of patients with symptoms due to ASD is twice as high as the rate of patients who will actually undergo repeated procedure for sASD [18, 20]. The mean NDI for ACDF and ACDF + CP in the present study was 14% which is slightly better than 21 and 22% reported in other studies [29, 31].

By comparing the clinical data of patients who were operated via ACDF to patients who were operated via ACDF + CP, no significant differences were seen postoperatively and at final follow-up. There was no significant difference regarding the rate of repeated procedure for DCDD and sASD between ACDF and ACDF + CP. Even though the rates for repeated procedure for DCDD and sASD were considerably lower after ACDF + CP (i.e., 4.8% and 2.4, respectively) compared to ACDF (i.e., 13.8 and 10.0%, respectively). However, adding of a cervical plate is believed to be a possible cause of postoperative dysphagia. Most recently, it has been reported that the postoperative dysphagia rate is about 8.5% (range 1.9–39.0%) following ACDF + CP [36]. At follow-up, none of

the patients reported to have dysphagia, even though in four patients (9.5%), removal of their cervical plate was performed due to chronic dysphagia. The reason of this extreme low rate of dysphagia at follow-up might be that no specific questionnaire for dysphagia assessment was performed. Further, all patients with documented dysphagia underwent repeated procedure. Also, it might be possible that patients have adapted to a mild form of dysphagia over the long postoperative course and therefore did not report it.

The authors want to state that the lower rate for repeated procedure after ACDF + CP might be influenced by the fact that the follow-up in these patients was significantly lower compared to patients who underwent ACDF.

In the past years, tremendous work has been done to identify factors for the development of degenerative changes at the adjacent segment. Concerns have been raised that increased rigidity by cervical fusion might be one of those factors [3, 28, 40]. The average annual incidence has been reported to be 2.9% for ACDF. The rate of patients with repeated procedure due to sASD is reported to be 11% within the first 5 years [21], 6.8–16% within the first 10 years [3, 20, 26], [21], and 16–33% of patients after up to 21 years postoperatively [19, 21]. Also, it has been reported that the risk of sASD is increased in 1–2 level procedures compared to 3 or more level procedures [24]. However, it still remains unclear whether 1 level is followed by a higher rate of procedure for sASD compared to 2 level procedures. In the present study the rate for repeated

procedure for sASD after 1 level ACDF and ACDF + CP and 2 level ACDF and ACDF + CP was 8.4, 0, 14.2, and 5.2% respectively. The overall rate for repeated procedure following 1 level and 2 level procedures was 6.4 and 10.0%, respectively. Based on this data, a longer fusion increases the risk of sASD which is in contrast to the aforementioned study.

Several studies indicate that the rate of annual incidence and repeated procedure after ACDF is higher compared to ACDF + PS. The annual incidence for the development of sASD after ACDF + CP has been reported to be 1.1–4.0%; the repeated procedure rate for sASD has been reported to be 1.3–1.8% within the first 2 years and 6.8–7.8% within the first 8 years [17, 32, 35, 38, 41, 44].

Lower reoperation rate for patients operated via ACDF + CP compared to ACDF has been presented previously [1]. Some authors believe that ACDF without plating might increase the risk of instability to the operated segment and that the adjacent segments are exposed to increased stress and degeneration might be accelerated. If ACDF without plating really increases the stress in the adjacent segments, sASD should occur within the first year till the index segment is fused. Since the majority of repeated procedure in this study and other studies were performed after 1 year, the authors believe that there is not sufficient data which supports this theory. Others might argue that instability at the index segment and increased stress at the adjacent segment increases the risk for developing a kyphotic alignment and that ACDF + CP offers better restoration of lordosis even in 1 level procedure [11, 22, 23]. In the authors' opinion, a major limitation in most of these studies is that diagnosis and age of patients are often not reported in detail. Patients with different diagnoses such as radiculopathy, myelopathy, trauma, tumor, or infection were treated with the same surgical technique [17]. A very low rate (1.3%) for repeated surgery at the adjacent segment due to newly developed symptoms was reported in a 24-month follow-up study by Selvanathan et al. Similar to our study, the surgical technique was an ACDF + CP; the rate of patients diagnosed with a CDH was 74%, and mean age at surgery was 48 years.

In contrast, Yue et al. reported a repeated surgery rate of 16.9% after ACDF + CP in a 7.2-year follow-up study. A closer look on diagnosis and patient's age at surgery supports the opinion of the authors that the development of sASD might be influenced by diagnosis and patient's age at surgery. In Yue's study, only 18.3% of patients had neural compression due to CDH. Further, the duration of symptoms prior to surgery was 27.2 months and therefore relatively long; the mean age at surgery was 52.7 years and considerably older compared to our cohort. In line with the study of Yue et al. are the results of Ishihara et al. who reported a similar rate (16%) for repeated procedure at the adjacent segment and similar mean age (51 years) at surgery in a 10-year of follow-up study [21]. There is no data on the issue whether the age at surgery

has an influence on the development of ASD. Hilibrand et al. stated that older patients had a higher degree of sASD whereas Lee et al. reported that patient age was not a significant risk factor for increased incidence of sASD [20] [24]. In the authors' opinion, age at initial procedure has an influence on the development of sASD because the degenerative process of cervical spondylosis at the adjacent segments is much more advanced in older patients. The rate of repeated procedure for sASD after ACDF and ACDF + CP in the present study is considerably lower compared to other authors (i.e., 4.9% within 10 years after ACDF). One reason might be that all patients were treated for a CDH which occurs in the early stages of cervical spondylosis. Another reason might be that the Anterior Cervical Trapezoidal Plate Stabilization System (by Aesculap, Tuttlingen, Germany) was a new surgical technique in the early 1980s and patient selection had been tighter these days [7].

Our rate of procedures per year was four- to sixfold smaller.

Further, sASD is influenced by the surgical technique, material for fusion, amount of fused levels, postoperative segmental alignment, and the personal life style of each patient. Some may argue that there is no difference in clinical outcome between ACDF and ACDF + CP and that ACDF + CP may be overtreatment and financially not viable. However, in the authors' opinion, a low postoperative complication rate and a lower repeated procedure rate favors ACDF + CP for the treatment of CDH in 1 to 3 level procedures.

Study limitations

Retrospective studies are often criticized for a low follow-up rate. In studies with a 10-year follow-up, a lost at follow-up rate of 50% or more is frequently reported.

Considering the fact that about 20% could not participate in the study due to death or poor physical condition, the actual follow-up rate in this report increases from 50 to 61%.

However, about one third of the contacted patients were not interested in participating in the study. The exact reason remains unclear, but this loss of patients is a bias to all of the presented outcome percentages.

Another limitation of this study is that the surgical technique of ACDF and ACDF + PS has evolved within the last decades. In the present study, ICDS morbidity was reported by 9.8% of all patients even after more than 20 years. To avoid this complication, the other majority of ACDF are performed using a machine-manufactured cage. Further, the modern anterior cervical plating systems have different biomechanical characteristics and are thinner compared to the original Caspar plate. These changes might have an effect on the postoperative course (i.e., dysphagia, material failure, cage subsidence). Additionally, it should be mentioned that only 226 patients fulfilled inclusion criteria in a period of more than 20 years. In the late 1990s, Savolainen et al. reported about

90 patients operated within 21 months, more recent studies by Xu et al. and van Eck et al. reported about 888 and 672 patients operated within 20 and 10 years, respectively [33, 41, 43]. Compared to the aforementioned studies, our rate of evaluated patients was four to sixfold smaller which might be a bias.

Conclusion

The rate of clinical success after ACDF and ACDF + CP for the treatment of CDH is 86.1% after more than 20 years of follow-up. The rate of patients who had no repeated procedure and who are free of radicular pain is 81.1%. The overall reoperation rate for degenerative changes at the cervical spine was 10.7%. There was no significant difference between ACDF and ACDF + CP according to clinical outcome, repeated procedure for DCDD, and symptomatic ASD.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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